

primary T-cells. **Conclusion:** We demonstrate an efficient gene silencing strategy in human primary T-cells. Thus, in addition to screening, identifying or verifying critical roles of various genes in T-cell functioning, GapmeR gene silencing technique may serve as a novel therapeutic modality in human diseases.

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**Board Number: B260**

**Elucidating Cellular Trafficking Pathways Controlling Prion-like Spread of Tau Aggregation Using CRISPR interference Screens.**

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Aggregation of Tau protein has been implicated in many neurodegenerative diseases such as Frontotemporal Dementia, and Alzheimer's Disease. The prion-like propagation of Tau aggregates is quickly emerging as a key mechanism driving disease progression. The cellular mechanisms controlling Tau aggregation and spread remain poorly understood. To reveal these mechanisms, we are leveraging a CRISPR interference (CRISPRi)-based platform for genetic screens that we have co-developed. CRISPRi enables highly specific knockdown of endogenous genes by programming a catalytically dead version of the dCas9 endonuclease fused to a transcriptional repressor domain with single guide RNAs. We model prion-like tau propagation by exposing a human FRET-based tau aggregation reporter cell line to pre-formed tau fibrils. In a genome-wide screen for factors controlling tau aggregation in this model, we identified several genes whose knockdown changed the extent of tau aggregation. Some of the most promising hits from our screen involve trafficking factors. Currently, we are testing the hypothesis that perturbation of specific trafficking pathways negatively or positively controls prion-like spreading of tau by inhibiting tau fibril uptake, intracellular trafficking, targeting to the lysosome, and export from the cell.

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**Board Number: B261**

**Reconstruction of a Genetic Pathway Using Transcriptome Mapping in a Metazoan.**

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RNA-seq is a technique that is commonly used to identify downstream elements to a genetic or chemical perturbation. In theory, global transcriptomes contain enough information to perform a full-fledged genetic analysis of a pathway. Early attempts to do this using microarrays in single-celled organisms reported partial reconstruction of a genetic pathway.

Using the hypoxia pathway, we show that genetic reconstructions can be performed using global transcriptome data. We obtained the transcriptomes of 5 *C. elegans* single mutants and 2 double mutants at the young adult stage. We show that in a blinded computational study, we were able to identify relevant genetic relationships between all genes in a pathway.

In addition, we identified a core set of ~500 genes that make up the bulk of the *C. elegans* hypoxia response. Multiple of these genes are directly implicated in the Electron Transport Chain (ETC), metabolism regulation or chemical damage responses.